

Search for Ion-Cyclotron Resonance in an Na^+ -Transport System

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Colonic tissue from the turtle (*Pseudemys scripta*) was exposed in a Ussing chamber to simultaneously applied static and time-varying magnetic fields. Transepithelial differences of potential were monitored as frequency of the AC field was varied continuously or in discrete steps from 3 to 770 Hz. Density of the DC field was varied from 10 to 220 μT and that of the AC field from 1 to 20 μT . Short-circuit currents through tissue were monitored for changes that might have been observed under ion-cyclotron resonance (ICR) conditions for each of several ions: H^+ , Li^+ , Na^+ , K^+ , Ca^{2+} , and Cl^- . No discernible changes of transepithelial current were observed. The negative findings are discussed in relation to positive and negative findings that have appeared in the literature.

Key words: ions, turtle, transepithelial currents

INTRODUCTION

There are now many published reports of the interaction of electromagnetic (EM) fields with a variety of biological systems. Of particular interest here are those that report that the interaction exhibits a "window" effect, a resonance-type of response to the frequency and amplitude of the EM field. Conti et al. [1983] report an inhibitory effect of the EM field on blastogenesis of mitogen-stimulated human lymphocytes, the inhibitory effect showing a "window" dependence on frequency (3–50 Hz). Delgado et al. [1982] reported that a pulsed EM field at 100 Hz and 1.2 μT had a powerful inhibitory effect on chicken embryogenesis. A "window effect" was also observed: Embryos exposed to fields of 100 Hz and 1.2 μT were less developed than embryos exposed at lower and higher intensities and frequencies.

Bawin and Adey (1976) have reported that weak, low-frequency sinusoidal electric fields modify the movement of calcium ions from freshly isolated chicken brains and cat cerebral tissue. Both frequency and amplitude windows were observed. Blackman et al. [1982] reported an enhancement in calcium-ion efflux from isolated chicken brains at specific ranges of low-frequency fields: 16 Hz fields were effective but 30, and 32 Hz fields were not. Although Blackman et al. [1982] reported an

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enhancement in calcium-ion efflux, Bawin and Adey (1976) reported a reduction. Blackman et al. [1985] suggested that the difference was due to Bawin and Adey's use of an oscillating (AC) electric field whereas Blackman et al. used an AC electromagnetic field. In testing this hypothesis, they demonstrated the importance of the AC magnetic component of the field, and, indeed, the importance of the local geomagnetic field.

The "window" effect led to the hypothesis of "ion-cyclotron resonance" (ICR) by Liboff [1985], which was subsequently extended by Chiabrera et al. [1985], who considered that the field acts on the binding process of a ligand to its binding site, a process occurring on the membrane's surface—the glycocalyx. Liboff, on the other hand, suggested that it is the transport of calcium and other ions through channels spanning the cell membrane that involves a resonance-type response to the applied EM field [Liboff, 1985; Liboff and McLeod, 1988]. The resonance is reported to occur in DC magnetic fields of the order of the geomagnetic field and in AC fields of the same magnitude and of frequency corresponding to the cyclotron frequency for a given ion in free space ($\omega_c = B q/m$, where q/m is the charge-to-mass ratio of the ion and B is the value of the magnetic field).

Some limited evidence does exist to support this idea. The harmonic content of the observed ICR effects in diatoms [McLeod et al., 1987] favors a channel-interaction site, as does the success of the calcium channel blocker nifedipine in cutting off the ICR effect in human lymphocytes [Liboff et al., 1987]. However, it is clear that a better controlled biophysical model involving channels in a more direct way will be required to test the ICR mechanism at the molecular level. One such attempt to observe resonances in Ca^{2+} transport in a variety of cells in culture by using the fluorescence indicator fura2 proved negative [Parkinson and Hanks, 1988]. A system involving a well-defined, oriented planar membrane is the turtle colon [Halm and Dawson, 1984], a sodium-absorbing epithelium in which sodium enters the cell across the apical membrane via sodium channels and exits the cell through an electrogenic pump that exchanges sodium for potassium. The ion pathways involved in this model are indicated in Figure 1. We report here on a second experiment yielding negative results, namely, a search for ICR effects on the total transmembrane ion current for turtle colon for H^+ , Li^+ , Na^+ , K^+ , Ca^{2+} , and Cl^- ions.

MATERIALS AND METHODS

Colons were removed from turtles (*Pseudemys scripta*) and stripped of serosal muscular layers as described elsewhere [Dawson, 1977] and mounted in lucite Ussing chambers with an exposed surface area of the membrane of 5.2 cm^2 . The chambers [Ussing and Zerahn, 1950] were equipped with two Ringer–agar bridges connected to calomel half-cells for the measurement of transepithelial potential difference (PD); two additional bridges connected to Ag–AgCl electrodes were employed to pass current across the tissue. The electrodes were connected to an electronic voltage clamp that could be adjusted to compensate for the solution resistance between the PD-measuring electrodes and the tissue surface. All measurements were made under short-circuit ($\text{PD} = 0$) conditions, and the short-circuit current (I_{sc}) and tissue conductance (G_T) were continuously monitored on a strip-chart recorder. Total G_T was measured as the change in current in response to a small change (10 m-V) in clamping potential. The duration of the 10 m-V pulse was varied from 0.5 to 20 s. Each side of

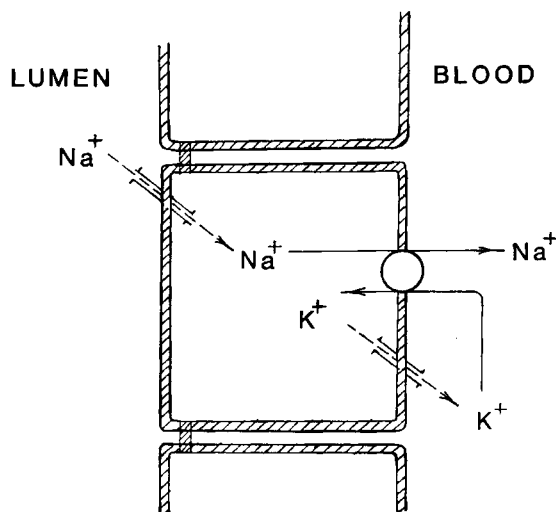


Fig. 1. The cellular mechanism of active sodium absorption by turtle colon. Sodium enters the cell across the apical membrane via Na^+ channels. Its exit from the cell is mediated by the $(\text{Na}^+ - \text{K}^+)$ ATPase pump, which exchanges 3 Na^+ for 2 K^+ .

the tissue was bathed in a solution. Three combinations were used: Ringer's-Ringer's, Ringer's-K-gluconate, and K-gluconate-K-gluconate. The Ringer's solution contained (in mM) Na, 114; Cl, 114; K, 2.5; HCO_3 , 2.5; Ca, 1.0; D-glucose, 5.0; and D-mannitol, 5.0. The K-gluconate solution was 112 mM.

The EM fields were applied by three pairs of Helmholtz-like coils mounted with respect to the Ussing chamber as shown in Figure 2. Each coil was wound on a Lucite form 5.5 inches (~ 14 cm) O.D., 4.75 inches (~ 12 cm) I.D., with a 0.25×0.25 inch ($\sim 0.65 \times 0.65$ cm) winding window. One pair, with 90 turns of No. 26 magnet wire on each form, was used to buck out the vertical component of the local geomagnetic field. The other two pairs, wound with 150 turns of No. 26 magnet wire on each form, were used to provide colinear DC and AC magnetic fields in the horizontal direction normal to the plane of the membrane. The axes of these coils (and of the Ussing chamber) were aligned with the direction of the horizontal component of the local geomagnetic field.

Constant currents for the DC magnetic fields were supplied by a Power Designs dual-current supply. Current for the AC field was supplied by a Crown amplifier driven by a Wavetek function generator to provide sinusoidal wave forms. The magnetic-field components, both DC and AC, were measured with a Bell Model-640 gaussmeter in conjunction with a Hall probe. The AC response of the gaussmeter extended from DC to an upper, half-power point of 5 kHz.

Observations consisted of looking for changes in the short-circuit current when the ICR field for a particular ion (H^+ , Li^+ , Na^+ , K^+ , Ca^{2+} , and Cl^-) was applied. The vertical component of the geomagnetic field was bucked out ($I = 51 \mu\text{A}$). The horizontal DC field was initially set at $50 \mu\text{T}$ ($I = 45 \mu\text{A}$) and the AC field at $50 \mu\text{T}$ —at the corresponding cyclotron resonant frequency as shown in Table 1.

The measurements were repeated with the horizontal DC field set to $B = 20 \mu\text{T}$ and the AC field set to $20 \mu\text{T}$ at each resonant frequency. The frequency was also

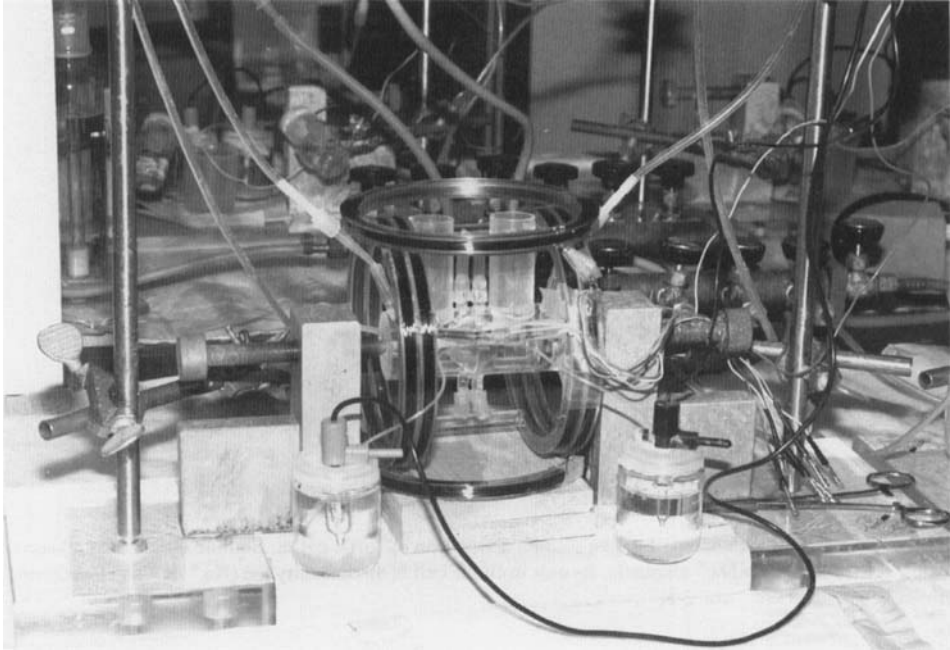


Fig. 2. Photograph of the experimental set-up. The plastic Ussing chamber is mounted between two colinear brass screws. The plane of the membrane is vertical and perpendicular to the local magnetostatic field. Three pairs of coils supply magnetic fields at the membrane that (1) buck out the local vertical component, (2) buck out the local horizontal component and add a net magnetostatic horizontal component, and (3) add an AC horizontal component parallel to the magnetostatic component.

varied, both continuously and in discrete steps from 3 to 770 Hz. The DC fields were varied from 10 to 200 μT and the AC field from 1 to 20 μT . The measurements were repeated on the same tissue sample the following day and also were repeated 1 week later on a tissue sample from the second turtle.

RESULTS

Transmembrane current was plotted as a function of time by a chart recorder, the baseline being the current measured via the current electrodes of the Ussing chamber with the voltage across the membrane clamped to zero. At intervals ranging from 2 to 20 s, a pulse at a potential difference of 10 mV was applied across the membrane for times ranging from 0.5 to 20 s, and the change in current was measured. If ICR alters the ion transport by opening conducting channels, then a change in either the baseline current or the current caused by the voltage pulse would be expected. Within the sensitivity of the present measurements, no change in current was detected when the ICR conditions were applied for the ions listed above, and no change was detected when the frequency and magnetic field were varied over the limits described above.

DISCUSSION

The lack of any discernible change in transepithelial current under the ICR conditions employed is in contrast with earlier reports [Thomas et al., 1986; Smith et

TABLE 1. Ion-Cyclotron Resonance Frequencies

Ion (% abundance)	Atomic weight (μ ; $1 \mu = 1.661 \times 10^{-27} \text{ kg}$)	q/m charge-to-mass ratio ([coul/kg] $\times 10^{-6}$)	Resonant frequency (Hz)			
			$B = 20 \mu\text{T}$		$B = 50 \mu\text{T}$	
H^+ (99.99)	1.008	95.6	304.00	760		
$^6\text{Li}^+$ (7.4)	6.015	C = 6.94	1.60	1.39	5.09	4.42
$^7\text{Li}^+$ (92.6)	7.016				12.7	11.1
$^{23}\text{Na}^+$ (100)	22.99		1.37		4.36	10.9
$^{24}\text{Mg}^{2+}$ (78.7)	23.99		4.19		13.3	32.2
$^{25}\text{Mg}^{2+}$ (10.1)	24.99	C = 24.32	8.04	7.92	25.6	64.0
$^{26}\text{Mg}^{2+}$ (11.2)	25.98				61.3	63.0
$^{35}\text{Cl}^-$ (75.33)	34.97	C = 35.46	7.70	7.42	24.5	25.2
$^{37}\text{Cl}^-$ (24.47)	36.97				61.3	63.0
$^{39}\text{K}^+$ (93.1)	38.96	C = 39.10	7.42	7.22	23.6	59.0
$^{41}\text{K}^+$ (6.88)	40.96				59.0	21.9
$^{40}\text{Ca}^{2+}$ (96.97)	39.96	C = 40.08	2.61	2.46	8.75	8.66
$^{42}\text{Ca}^{2+}$ (0.64)	41.96				21.9	21.7
$^{43}\text{Ca}^{2+}$ (0.145)	42.96	C = 39.10	2.61	2.46	8.31	20.8
$^{44}\text{Ca}^{2+}$ (2.06)	43.96				20.8	19.6
$^{48}\text{Ca}^{2+}$ (0.18)	47.95	C = 40.08	2.47	2.46	7.86	7.83
$^{45}\text{Ca}^{2+}$	44.95				19.7	19.6
			2.35		18.7	
			4.82		15.3	38.4
			4.59		14.6	36.5
			4.48	4.81	14.3	15.3
			4.38		13.9	37.8
			4.02		34.9	38.2
			4.29		32.0	
					34.2	

$f = B/2\pi \cdot q/m$.

C, chemical atomic weight.

al., 1987; Liboff et al., 1987] in which biological changes were observed at predicted cyclotron-resonance frequencies that corresponded to magnetic fields of the order of the geomagnetic field ($\sim 50 \mu\text{T}$). The present negative results are, however, consistent with one other experiment that was designed to test for the ICR phenomenon [Parkinson and Hanks, 1988]. Possible explanations for this discrepancy are:

1. The two experiments that yielded negative results measured only a short-term response, the exposures varying between 2 and 20 min. The exposures for the experiments yielding positive results were relatively long and might have been sensitive to an integrated effect.

2. The membrane employed in the present experiment may involve ion-transport mechanisms different from those in which the ICR phenomenon has been observed. For example, the positive ICR reports listed above utilized magnetic field combinations specific to calcium transport. The turtle-colon model is a sodium-transport system.

3. The technique employed with the turtle colon measures the net current across the epithelium and not the cellular transmembrane current. Charges may flow through paracellular pathways (acting via tight junctions) and also by means of counterion transport external to both the apical and basal membranes. If the ICR enhances ion transport only across cellular membranes, then the sensitivity of the present experiment might not be adequate.

4. In the diatom experiment [Smith et al., 1987] and in the lymphocyte experiment [Liboff et al., 1987], it was necessary that the extracellular calcium concentrations be low to observe the ICR response. No special attempt was made in the present experiment to reduce the external ionic concentrations, with the exception of the potassium-reduced (2.5 mM) Ringer's solution used in one measurement.

Finally, we note that the negative results permit an upper limit to be set on the number of channels that have their conductance altered via the ICR mechanism. The lower limit on current sensitivity is estimated to be $0.2 \mu\text{A}/\text{cm}^2$. Given the sample area of 5.2 cm^2 and the approximate cross-sectional area of each cell ($400 \mu\text{m}^2$), then the total number of cells involved is approximately 1.3×10^6 . Although the density of channels is not well known and does vary with a number of factors, an estimate of ~ 100 channels/ (μm^2) is a reasonable guess [Tedeschi et al., 1987]. This implies 4×10^4 channels/cell, and thus approximately $1.3 \times 10^6 \times 4 \times 10^4 = 5 \times 10^{10}$ channels within the sample area. The minimal current that could have been detected per channel was therefore 3.2×10^{-16} amperes, or approximately 100 ions/s/channel if all channels were simultaneously affected or perturbed by the ICR condition. Normal ionic fluxes are estimated to be 10^7 ions/s/channel [Urry et al., 1980; Darnell et al., 1986] for a 1 M external to 10^{-7} M internal concentration gradient. Assuming a flux of 10^4 ions/s/channel for 10^{-3} external concentration, a conservative estimate that assumes a linear response to molarity, then only one channel in 10^2 would have to be affected by ICR for an effect to be detected. It should be noted also that the response would be rapid, on the order of 1 s or less.

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